ORAL DELIVERY OF VACCINES USING POLYMERIZED LIPOSOMES

This is a continuation of application Ser. No. 08/096,689, filed Jul. 23, 1993, now abn.

This invention relates to the synthesis, preparation, and use of polymerized liposomes as sustained drug and/or antigen release devices.

BACKGROUND OF THE INVENTION

Drug delivery takes a variety of forms, depending on the agent to be delivered and the administration route. The most convenient way to administer drugs into the body is by oral administration. However, many drugs, in particular proteins and peptides, are poorly absorbed and unstable during passage through the gastrointestinal (G-I) tract. The administration of these drugs is generally performed through parenteral injection.

Although oral vaccination is more convenient, vaccines are generally given through injection. This is particularly true with killed or peptidic vaccines, because of their low absorbability and instability in the G-I tract. A problem with systemic immunization is that it may not effectively induce mucosal immune responses, particularly production of IgA, that are important as the first defense barrier to invaded microorganisms. For this reason, it would be beneficial to provide oral vaccination, if the problems of low absorbability and instability could be overcome.

Controlled release systems for drug delivery are often designed to administer drugs to specific areas of the body. In the gastrointestinal tract it is important the drug not be eliminated before it has had a chance to exert a localized effect or to pass into the bloodstream.

Enteric coated formulations have been widely used for many years to protect drugs administered orally, as well as to delay release. Several microsphere formulations have been proposed as a means for oral drug delivery. For example, PCT/US90/06430 and PCT/US90/06433 by Enzytech discloses the use of a hydrophobic protein, such as zein, to form microparticles; U.S. Pat. No. 4,976,968 to Steiner, et al. discloses the use of "proteinoids" to form microparticles; and European Patent Application 0.333,523 by The UAB Research Foundation and Southern Research Institute discloses the use of synthetic polymers such polylactic acid-glycolic acid to form microspheres.

Particles less than ten microns in diameter, such as the microparticles of EPA 0.333,523, can be taken up by cells in specialized areas, such as Peyer's patches and other intestinal mucosal lymphoid aggregates, located in the intestine, especially in the ileum, into the lymphatic circulation. Entrapping a drug or antigen in a microparticulate system can protect the drug or antigen from acidic and enzymatic degradation, yet still allow the drug or antigen to be administered orally, where they are taken up by the specialized uptake systems, and release the entrapped material in a sustained manner or are processed by phagocytic cells such as macrophages. When the entrapped material is a drug, elimination of the first-pass effect (metabolism by the liver) is highly advantageous.

Liposomes have been proposed for use as an oral drug delivery system, for example, by Patel and Ryman, *FEBS Letters* 62(1), 60–63 (1976). Liposomes are typically less than 10 microns in diameter, and, if they were stable to passage through the GI tract, should be absorbed through the Peyer's patches. Liposomes also have some features that should be advantageous for a particulate system for oral drug or antigen delivery. The phospholipid bilayer membrane of liposomes separates and protects entrapped materials in the inner aqueous core from the outside. Both water-soluble and -insoluble substances can be entrapped in different compartments, the aqueous core and bilayer membrane, respectively, of the same liposome. Chemical and physical interaction of these substances can be eliminated because the substances are in these different compartments. Further, liposomes are easy to prepare. However, liposomes are physically and chemically unstable, and rapidly leak entrapped material and degrade the vesicle structure. Without fortifying the liposomes, they are not good candidates for oral drug or antigen delivery.

Several methods have been tried to fortify liposomes. Some methods involved intercalating cholesterol into the bilayer membrane or coating the liposome with polysaccharides. These methods are not useful in making liposome for oral delivery since during oral delivery liposomes are exposed to an acidic pH in the stomach and bile salts and phospholipases in intestine. These conditions break down the cholesterol and polysaccharide in the liposomes.

There remains a need for drug and antigen delivery devices that can survive the harsh conditions in the GI tract, and yet effectively deliver the drug and antigen.

It is therefore an object of the invention to provide stable liposomes for use in oral drug and antigen delivery.

It is a further object of the invention to provide methods of preparing stabilized liposomes.

It is still a further object of the invention to provide a method for orally administering drugs or antigens entrapped within these stabilized liposomes to a patient in need of the drug or antigen.

SUMMARY OF THE INVENTION

Polymerized liposomes are disclosed which are useful in oral delivery of compounds. The constituent phospholipids and/or the leaflets are polymerized by covalent bonding to each other. Covalently binding the layers adds strength, resulting in a less fluid unpolymerized liposome. The less fluid bi-layer membrane suppresses leakage. Further, the detergent-like bile salts in the intestine cannot extract the phospholipid molecules. These cross-linked membranes are strong enough to maintain their structure even if the phospholipids undergo hydrolysis at low pH and enzymatic degradation by phospholipases. Polymerized liposomes reach the ileum of the GI tract as intact particulates, and are absorbed.

Polymerized liposomes are prepared by polymerizing double bond-containing olefinic and acetylenic phospholipids. In addition, polymerized liposomes can be prepared by chemical oxidation of thiol groups in the phospholipids to disulfide linkages. The polymerization can take place in a solution containing a biologically active substance, such as a drug or antigen, in which case the substance is encapsulated during the polymerization. Alternatively, the liposomes can be polymerized first, and the biologically active substance can be added later by resuspending the polymerized liposomes in a solution of a biologically active substance, and entrapping the substance by sonication of the suspension. Another method of entrapping a biologically active substance in polymerized liposomes is to dry the polymerized liposomes to form a film, and hydrate the film in a solution of the biologically active substance. The above conditions are typically mild enough to entrap biologically active substances without denaturing them.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of the percent BSA (Bovine Serum Albumin) released from polymerized liposomes of DODPC